## IMPACTS OF METHYL BROMIDE AND ITS ALTERNATIVES ON SOIL MICROBIAL COMMUNITIES

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Soil fumigation is the primary method used in reducing soilborne plant pathogens and parasitic nematodes that cause severe damages to high value crops in the warm region of the U.S. It is often required to reduce root-knot nematode populations. The fumigants methyl bromide (MeBr), 1,3-dichloropropene (1,3-D), methyl isothiocyanate (MITC), and chloropicrin are known to have broad biocidal activity, and their effects on soil bacteria are largely unknown. Recently, the effect of MITC (the toxic degradation product of metam sodium) on soil bacterial population structure and function was studied by the use of traditional heterotrophic activity measures, and biochemical assays.

The long-term goal of the research in our laboratory in response to the EPA action is to evaluate new or existing alternative fumigants for control of soilborne plant pathogens to replace methyl bromide in high value crop areas. Biologically-based and environmentally-safe alternatives, such as compost amendment and biosolid application, are being investigated for possible use in integrated management strategies for accelerated degradation of fumigants. The hypothesis governing this practice is that organic amendments, when applied to soil, add different substrates that can be used by soil bacteria for growth. However, soils are fumigated by direct injection into the subsurface soil with little or no surface litter. The impacts of this practice on soil microorganisms have rarely been shown. The impact of fumigants on soil microorganisms is being evaluated in view of their role in sustaining the global cycling of matter and their function in supporting soil quality for productive agriculture. In this paper, we describe the effect of MeBr, MITC, 1,3-D and chloropicrin on soil microbial population in a laboratory microcosm experiment. Our objectives were to monitor the biocidal effects of these fumigants and compare their ecotoxicological effects on soil microorganisms in response to fumigation at recommended application rates.

To overcome the drawbacks of studying the effect on individual bacterial strains, since only about 1% of total bacteria in the soil can be cultured, we used the nucleic acid approach by employing different PCR methods to analyze the total bacterial population in our samples. We also used biochemical and metabolic approaches to evaluate how microorganisms respond to certain carbon substrates after fumigation. We used the Biolog system in this study to monitor functional changes by heterotrophs and PLFA/DNA fingerprinting to quantify microbial biomass and community diversity composition. Since microbial biomass as determined by PLFA is based on the relationship between the phylogeny of microorganisms and their PLFA profiles, we believe that the use of PLFA techniques provides an unbiased description of the effects of fumigants on microbial population. This allowed us to interpret the effect of fumigants on different groups of bacteria, fungi, and actinomycetes. The total bacterial DNA fingerprinting by denaturing gradient gel electrophoresis (DGGE) provided a complete picture on the effects of these compounds on the dominant bacterial populations. Through this we calculated the structural diversity of the microbial community. This provided distinct diversity value for each sample and we were able to observe changes in bacteria composition over the 12 week study period.

Soil samples (Arlington sandy loam) were taken from the top 15 cm in a field from the University of California, Riverside Agricultural Experiment Station. There has been no history of fumigant treatments on this plot. Soil microcosms consisted of glass jars containing about 1 kg soil (dry wt). Fumigants were added as freshly prepared aqueous solutions sufficient to bring soil moisture contents to about 1.8 kg kg-1 dry soil. MrBr and MITC were applied at 0, 160, 320, and 3200 mg kg-1 dry soil, 1,3-D at 0, 80, 160, and 1600 mg kg-1, and chloropicrin at 0, 40, 80, and 800 mg kg-1 dry soil. Microcosms were sealed for 24 h after fumigant application and were vented continuously thereafter. Samples for testing of microbial activity were taken after 1, 8, and 12 weeks.

Major bacterial populations were below detection levels for all MeBr treatments after the first week. The same effects were observed at the highest concentration of 1,3-D and chloropicrin. After 12 wk there was a significant recovery of major species in the soil as revealed by more intense banding pattern. There were no significant differences between our control soils and the three other fumigants after 8 weeks incubation. After 12 weeks incubation, there were distinct shifts in both the species composition and relative abundance of different bacterial groups. The Shannon index of diversity H was calculated for each fumigated soil sample. High diversity index was maintained for the control soil and the fumigated soils, except those treated with methyl bromide (H decreased from 1.11 to 0.13). After 12 weeks incubation, H increased to 0.63 in the methyl bromide treated samples.

In summary, this laboratory experiment supports the effectiveness of MeBr as a soil fumigant. It shows that MeBr does not only kill fungi or nematodes, but other soil microorganisms at the time of application. It also demonstrates that after a few weeks, soil bacteria will recover by using MeBr as substrate for growth. It also showed that MITC and 1,3-D and chloropicrin had very little effect on soil microorganisms. Therefore, application of MITC and 1,3-D and chloropicrin as alternatives to MeBr may maintain soil health while controlling pathogenic organisms.